Effect of pulsed electric fields on the antioxidant potential of apples stored at different temperatures

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Abstract

The effects of pulsed electric fields (PEF, 0.008 – 1.3 kJ kg^{-1}) on the total phenolic, flavonoid and flavan-3-ol contents, as well as on the antioxidant capacity of apples stored at different temperatures (4 and 22 ºC) along 48 h were studied. Contents of phenolic compounds observed in PEF-treated apples were higher than those of untreated. The mildest PEF treatment (0.008 kJ kg^{-1}) produced the maximum increases of total phenolics (13 %) and flavan-3-ol (92 %) contents in apples stored during 24 h at 22 ºC, while it was observed at 4 ºC for flavonoids (58 %). On the other hand, the antioxidant capacity of apples was enhanced by 43 % respect to that of untreated with the mildest PEF treatment after 12 h at 4 ºC and by 15 % after 24 h at 22 ºC. Therefore, PEF technology could be used to increase the antioxidant potential of apples by controlling treatment and storage conditions.

Keywords

Apple; pulsed electric fields; phenolic compounds; antioxidant capacity; abiotic stress

1. Introduction

Consumers are more and more concerned about the nutritional and health-related characteristics of fruits and vegetables. Evidence suggests that a diet high in fruits and vegetables may decrease the risk of chronic diseases because of their high content in phytochemicals (Boyer and Liu, 2004). Apples are among the most popular and frequently consumed fruits in the world, because of their availability throughout the year and the general perception that apples are good for health. Epidemiological studies support the view that frequent apple consumption is associated with a reduced risk of chronic pathologies such as cardiovascular disease, specific cancers, and diabetes (Koutsos et al., 2015). The health benefits of apple consumption are mainly related with phenolic compounds content (Hyson, 2011). Moreover, there is a strong correlation between phenolic content of apples and antioxidant activity (Kalinowska et al., 2014). The antioxidant compounds in some fruits and vegetables can be lost during handling after harvest, even during minimal processing and storage. In this sense, postharvest treatments are needed to preserve the quality and antioxidant potential of fresh produce (Villa-Rodriguez et al., 2015). The application of postharvest abiotic stresses (i.e., wounding, UV-light radiation, modified atmospheres, exogenous phytohormones) has been proposed in recent years as an effective strategy to activate the secondary metabolism of fruits and vegetables leading to the accumulation of antioxidant compounds with health-promoting benefits (Becerra-Moreno et al., 2015). Some reports suggest that pulsed electric fields (PEF) could act as abiotic stressor when applied during postharvest affecting the metabolism of vegetables (Galindo et al., 2008; Galindo et al., 2009).

62 PEF technology has been extensively studied as preservation technique of foods.
63 Numerous studies have demonstrated the ability of PEF to obtain shelf-stable plant-
64 based liquid foods with high nutritional and sensory value (Odriozola-Serrano et al.,
65 2013; Saldaña et al., 2014). Moreover, PEF may also be used as a pretreatment of solid
66 vegetable matrices to improve processes such as extraction by pressing or solvent
67 diffusion, osmotic dehydration, drying, and freezing (Donsi et al., 2010).
68 Recently, PEF has been proposed as a promising new abiotic elicitor for stimulating the
69 secondary metabolites biosynthesis and accumulation in plant cell cultures (Cai et al.,
70 2011; Gueven and Knorr, 2011; Saw et al., 2012). Little information has been found in
71 the scientific literature regarding the use of PEF as possible treatment to enhance or
72 stimulate the production of sencondary plant metabolites, such as phenolics, in fruits
73 and vegetables. Vallverdú-Queralt et al. (2012) observed a maximum increase in total
74 phenolics content (36.6 %) when tomato fruits were stored at 4 ºC for 24 h after a PEF
75 processing of 1 kV cm^{-1} and 16 pulses, contributing to an increase in the antioxidant
76 capacity of tomato fruit by more than 20 %. Vallverdú-Queralt et al. (2013) reported
77 that 24 h at 4 ºC after PEF treatments (0.4 to 2.0 kV cm^{-1} and 5 to 30 pulses) led to an
78 increase in hydroxycinnamic acids and flavanones contents in tomato fruits, whereas
79 flavonols, coumaric and ferulic acid-O-glucoside were not affected. Moreover, the
80 increases of phenolic compounds concentrations depended on the PEF treatment
81 intensity. However, as far as we know, no information is available regarding the effects
82 of PEF on the antioxidant potential of fruits and vegetables stored at different
83 temperatures. Therefore, the aim of this work was to evaluate the impact of PEF
84 treatment intensity (0.008 – 1.3 kJ kg^{-1}) on the phenolic compounds content and the

antioxidant capacity of apples stored at different temperatures (4 and 22 ºC) during 48 h.

2. Material and Methods

2.1. Reagents

Methanol (HPLC grade), Folin–Ciocalteu reagent (2 N), hydrochloric acid 37 % and sodium hydroxide were purchased from Scharlab S.L (Sentmenat, Spain). Sodium carbonate was obtained from POCH S.A (Poland). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, sodium nitrite 97 % and (+)-catechin were purchased from Sigma Aldrich Co. (St. Louis, MO, EUA). Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) 97 % was supplied by Sigma Aldrich Chemie GmbH & Co. KG (Steinheim, Germany). Vanillin 99 % and aluminum chloride were purchased from Acros Organics (New Jersey, USA). High-purity water (Milli-Q water) was produced in the laboratory (Millipore Corporation, Bedford, MA, USA).

2.2. Sample preparation

Commercially mature apples (*Malus domestica*, var. Golden delicious) were purchased from a local supermarket (Lleida, Spain). The fruits were kept under regular cold storage until processing without applying any postharvest treatment. Apple fruits were selected according to uniformity in maturity and sanity. The pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), the titratable acidity, the soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan), the colour

(Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) and the firmness (TA-XT2 Texture Analyzer, equipped with a 4 mm rod, Stable Micro Systems Ltd., Surrey, England, U.K.) of apples were determined. The physic-chemical characteristics of apples were: pH = 3.90 ± 0.11, titratable acidity = 4.55 ± 0.85 g L^{-1} malic acid, soluble solids = 13.35 ± 0.07 %, colour: L* = 73.63 ± 2.01, a* = -15.20 ± 2.47 and b* = 43.49 ± 0.50, and firmness = 7.52 ± 0.54 N. Apples were washed with chlorinated water (200 mg L^{-1}) for 5 min before use.

2.3. PEF processing of apples

PEF treatments were conducted in a batch equipment (Physics International, San Leandro, CA, USA) which delivers pulses from a capacitor of 0.1 μF with an exponential decaying waveform. A stainless steel parallel plate (20x8 cm) treatment chamber with a distance between plates of 10 cm was employed, using tap water as conductive medium. Whole apple fruits (two per batch) were treated at 0.4 – 2 kV cm^{-1}, using 5 – 35 monopolar pulses of 4 μs at a frequency of 0.1 Hz, which correspond to an specific energy input of 0.008 – 1.3 kJ kg^{-1}. PEF-treated and untreated apples were stored at different temperatures (4 and 22 ºC) and times (0, 12, 24, 36 and 48 h). After each storage time, samples were freeze dried and kept at -30 ºC until analysis.

2.4. Phenolics and antioxidant capacity analysis

2.4.1. Phenolics extraction

The extraction of phenolics was based on the methodology followed by Patras et al. (2009) with some modifications. Methanolic extracts were prepared by adding 1 g of

freeze dried samples to 5 mL of 80 % methanol and homogenizing for 2 min at 13,600 rpm using an Ultra-Turrax T 25 (IKA® WERKE, Germany). The samples were then centrifuged for 20 min at 4020 x g and 4 ºC (Hettich® EBA 21 centrifuge, Andreas Hettich GmbH & Co.KG., Tuttlingen, Germany) and filtered through Whatman No 1 filter paper. The supernatant was transferred into a volumetric flask. The extraction of the residue was repeated adding 5 mL of 80 % methanol, sonicating for 5 min and centrifuging for 20 min at 4020 x g and 4 ºC. Both supernatants were combined into the same volumetric flask. The resulting methanolic extract was used to determine the total phenolic, flavonoid and flavan-3-ol contents as well as the total antioxidant capacity.

2.4.2. Determination of total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu reagent according to the method of Odriozola-Serrano et al. (2008). A portion of 0.5 mL of methanolic extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 10 mL of saturated Na_2CO_3 solution. Samples were mixed and stored at room temperature in darkness for 60 min. Absorbance was measured at 725 nm using a CECIL 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Calibration curve was built with gallic acid (0-300 mg L^{-1}). Results were expressed as grams of gallic acid equivalents (GAE) per kilogram.

2.4.3. Determination of flavonoid content

Flavonoid content was determined based on the method described by Dávila-Aviña et al. (2012) with some modifications. One milliliter of the methanolic extract, 4 mL of deionized H_2O and 0.3 mL of $NaNO_2$ (5 %) were mixed in a volumetric flask (10 mL).

157 After 5 min, 0.3 mL of $AlCl_3$ (10 %) were added and stored in the darkness for 1 min.
158 Two milliliters of NaOH (1 mol L^{-1}) were added and the volumetric flask was adjusted
159 by adding deionized H_2O. The absorbance was determined at 478 nm using a CECIL
160 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Calibration curve
161 was built with (+)-catechin (0-300 mg L^{-1}). Results were expressed as grams of (+)-
162 catechin equivalents (CE) per kilogram.
163
164 2.4.4. Determination of flavan-3-ol content
165 Flavan-3-ol content determination method was based on the vanillin assay described by
166 Carbone et al. (2011) with some modifications. A volume of 1 mL of the methanolic
167 extract and 5 mL of vanillin (1 %) in methanol were mixed and rested for 5 min, and
168 then, 5 mL of HCl (4 %) were added. The absorbance was measured at 494 nm after 20
169 min of reaction time at room temperature using a CECIL 2021 spectrophotometer (Cecil
170 Instruments Ltd., Cambridge, UK). Flavan-3-ol content were calculated from a
171 calibration curve, using (+)-catechin (0-1500 mg L^{-1}) as standard. Results were
172 expressed as grams of (+)-catechin equivalents (CE) per kilogram.
173
174 2.4.5. Determination of antioxidant capacity
175 The method used to measure the total antioxidant capacity was based on the DPPH
176 assay described by De Ancos et al. (2002). Briefly, 0.05 mL of the methanolic extract or
177 trolox standard, 0.05 mL of Milli-Q water and 3.9 mL of methanolic DPPH (0.025 g L^{-1})
178 were mixed, shaken and left in the dark for 30 min at room temperature. The
179 absorbance was measured at 515 nm using a CECIL 2021 spectrophotometer (Cecil
180 Instruments Ltd., Cambridge, UK) against a blank of methanol without DPPH. A

calibration curve was obtained with the percentage of inhibition of the DPPH as a function of trolox standard concentration (0 - 0.4 mg mL^{-1}). Results were expressed as mmol of trolox equivalents (TE) per kilogram.

2.5. Statistics and experimental design

Two replications of each treatment were carried out and samples were analysed in triplicate. A multifactor analysis of variance (ANOVA) was performed at $p<0.05$ in order to assess phenolic compounds content and antioxidant capacity changes among the PEF treatment intensities (0, 0.008, 0.3 and 1.3 kJ kg^{-1}), storage temperatures (4 and 22 ºC) and times (0, 12, 24, 36 and 48 h). This statistical analysis was performed using the software JMP Pro 11 (SAS Institute Inc., Buckinghamshire, UK).

3. Results

3.1. Total phenolic content

Changes in total phenols as affected by PEF treatment intensity, storage time and temperature can be observed in Figure 1. The initial phenolic content of fresh untreated apples ranged from 3.1 to 3.2 g kg^{-1} of GAE, whereas PEF-treated apples exhibited values in the range of 2.6 – 3.2 g kg^{-1} of GAE. It can be deduced that the application of PEF treatments with different intensity, characterized by their specific energy input, did not lead to major changes in total phenolic compounds just after processing. Although mean values in some PEF-treated samples were slightly lower, a significant effect of the treatment could not be drawn ($p>0.05$).

205 Pooled data indicate that the total phenolic content of apples was affected by storage
206 time and temperature, as well as by the interaction of these factors with the applied PEF
207 treatment conditions ($p<0.01$). Untreated apples exhibited stable phenolic contents over
208 48 h regardless the storage temperature, while the application of PEF led to divergent
209 results, depending on the specific energy of the treatments and the storage temperature.
210 On the one hand, a dramatic drop in total phenolic content occurred during the 12 h
211 following the application of PEF at 1.3 kJ kg^{-1} and continued to decrease subsequently.
212 This decrease accounted for more than a 50 percent of the initial phenolic content over a
213 48-h period. On the other hand, an increase in the total phenolic content could be
214 observed in apples subjected to the mildest PEF treatment conditions (0.008 kJ kg^{-1})
215 during the day following processing. Thus, the highest phenolic content of 4.3 g kg^{-1} of
216 GAE was found in apples stored for 24 h at 22 ºC (Figure 1B). This value represents a
217 35 % increase compared to the initial content just after treatment and a 13% increase
218 compared to the content found in untreated apples kept under the same storage
219 conditions.
220
221 3.2. Flavonoid content
222 Flavonoids were the main polyphenolic constituents in apples, with an average content
223 within the range of 83 – 91 % in the fresh untreated fruits (Figure 2). PEF treatments
224 did not cause significant modifications of the flavonoid content, at least immediately
225 after processing. Hence, the highest initial flavonoid content (2.9 g kg^{-1} of CE) was
226 found in apples treated under the most intense conditions (1.3 kJ kg^{-1}). Temperature was
227 not found to play a significant role in the flavonoid content of just treated apple fruits
228 ($p>0.05$). In contrast, storage time and temperature significantly ($p<0.01$) affected the

229 flavonoids content. This effect significantly ($p<0.01$) differed among PEF treatments.
230 Hence, on the one hand, flavonoid concentrations were maintained or even increased
231 over a 48 h period in either untreated or mildly treated (0.008 kJ kg^{-1}) apples, whilst, on
232 the other hand, more intense treatments led to a significant decrease through storage. In
233 the former case, flavonoids sharply increased following the application of PEF
234 treatments with a specific energy input of 0.008 kJ kg^{-1}. The highest flavonoid
235 concentrations in absolute terms (3.4 – 3.6 g kg^{-1} of CE) were observed at 24 h after that
236 treatment regardless the storage temperature. However, storage for 24 h at 4 ºC resulted
237 into the greatest accumulation of flavonoids in relative terms, which was 58 % above
238 the average value observed in untreated apple fruits (Figure 2A). In the latter case, the
239 delivery of specific energy inputs of 0.3 and 1.3 kJ kg^{-1} resulted into an average loss of
240 41 and 67 % of the flavonoid content, respectively, with respect to the concentrations
241 found in untreated apples 48 h after the treatment.
242
243 3.3. Flavan-3-ol content
244 The effect of PEF on the accumulation of flavan-3-ol in apple fruits is shown in Figure
245 3. The initial contents ranged between 0.7 and 0.8 g kg^{-1} of CE. On average, these
246 values represented a 26 % of the estimated total phenolic content in apple fruits. At a
247 first stage, the treatments did not apparently affect the concentration of flavan-3-ol
248 compounds, provided that neither correlations between treatment intensity and initial
249 contents nor significant ($p<0.05$) differences between the contents in treated and
250 untreated fruits could be established. However, the flavan-3-ol concentrations
251 subsequently changed, thus revealing a dynamic relationship between PEF treatment
252 intensity, storage time and temperature. Storage temperature was the main factor

253 significantly influencing the synthesis or destruction of flavan-3-ol compounds over
254 storage. Hence, the concentrations of these flavan derivatives substantially differed
255 between apples stored under refrigeration and ambient temperatures. Flavan-3-ol
256 compounds in untreated fruits did not substantially change through storage at 4 ºC
257 (Figure 3A). At that same temperature, a maximum peak concentration of 0.9 g kg^{-1} of
258 CE was reached at 24 h following the mildest PEF treatment (0.008 kJ kg^{-1}), and
259 subsequently declined. Significant downward trends could be observed for more intense
260 PEF applied conditions. In this regard, a dramatic loss of flavan-3-ol content was
261 observed in apple fruits subjected to the most intense treatments. This depletion
262 remained consistent over time and resulted in a 75 % lower flavan-3-ol concentration
263 than that found in untreated apple fruits at 48 h after the PEF treatment. Overall, the
264 trends observed for the changes in flavan-3-ol concentrations in apples stored under
265 different temperatures (4 and 22 ºC) were similar, although the extent of the changes
266 was substantially different. The rate of increase in flavan-3-ol compounds during 24 h at
267 22 ºC following the mildest PEF treatment was 8-fold higher than that in the fruit
268 treated at the same conditions and stored under refrigeration, thus reaching a maximal
269 concentration, in absolute terms, of 2.0 g kg^{-1} of CE. This amount was a 92% higher
270 than the concentration found in untreated apples stored under the same time-temperature
271 conditions.
272
273 3.4. Antioxidant capacity
274 Figure 4 shows changes in the antioxidant capacity of apple fruits, measured through
275 the DPPH assay, as affected by PEF treatment and storage conditions. Interestingly,
276 PEF treatments significantly ($p<0.01$) promoted the overall antioxidant capacity of

277 apples. The fruits treated with a PEF treatment delivering 0.3 kJ kg^{-1} exhibited highest
278 increases of 2.1- and 1.4-fold for antioxidant capacity as compared with the untreated
279 fruits at 4 and 22 ºC, respectively. As well, the changes in antioxidant capacity
280 throughout storage time were strongly determined by the intensity of the PEF treatment.
281 However, the sign of the subsequent effects substantially differed from those observed
282 in the just-treated fruits. Consequently, despite the initial rise observed in the fruits
283 subjected to the most intense PEF treatment (1.3 kJ kg^{-1}), the antioxidant capacity
284 dramatically dropped during the 12-24 h following the treatment. In contrast, milder
285 PEF conditions led to an abrupt increase in the antioxidant capacity of apples. This
286 increase reached its summit in fruits treated under the mildest PEF conditions (0.008 kJ
287 kg^{-1}). Namely, the antioxidant capacity of apple fruits treated and stored at 4 ºC
288 underwent a 2.4-fold rise during the first 12 h of storage and took a maximal value of
289 2.9 mmol TE kg^{-1} (Figure 4A), which represents a 43% increase over the untreated
290 samples at that time. Storage at 22 ºC led to a similar trend, although the peak value for
291 antioxidant capacity was observed 24 h after the application of the PEF treatment
292 (Figure 4B).
293
294
295 **4. Discussion**
296 Results indicate that apple fruits exposed to mild PEF conditions exhibited a significant
297 increase of their phenolic content, which took place after the treatment application.
298 Although the increase in total phenolic content may seem difficult to explain due to the
299 complexity of biological systems, a well-recognized explanation for these observations
300 is the production of polyphenolic metabolites through the phenylpropanoid metabolism,

known to be part of the plant defence response against oxidative stress (Dixon and Paiva, 1995; Amodio et al., 2005). However, it is not easy to establish direct relationships between the treatment conditions and the tissue response, which is supported by the fact that no straightforward correlations could be established between specific energy inputs and the accumulation of phenolic compounds in the tissue. Although the extent of the response observed seems to be conditioned by multiple factors, the intensity of the PEF treatment appears to stand as a critical aspect. PEF treatments result in the formation of pores in the plasma membrane, which may result in the efflux and influx of ions and other polar constituents. The observations by Galindo et al. (2008) support this statement, providing evidence of a fast metabolic response upon the application of PEF treatments to potato tissues and the production of reactive oxygen species (ROS) in the wounded tissues. Our results show that phenolic compounds were more likely to accumulate in the fruits treated under the mildest PEF treatment conditions. This strongly suggests that the extent of the metabolic response is determined by the size and persistence of the generated pores. As small scale pores are more likely to possess a transient nature and reseal in extremely short times, in the order ns (Ji et al., 2006), the metabolic work and amount of energy required by the tissue for resealing and further recovery after the application of mild PEF conditions would explain the greater increase in phenolic content and overall antioxidant capacity observed in the present study. In a previous work, the production of ROS in *Taxus chinensis* suspension cultures and an enhancement of secondary metabolite accumulation in the tissue cells were reported to be affected by the changes in the cell/membrane's dielectric properties, which in turn depended on the intensity and the exposure time (Ye et al., 2004). Authors concluded that the changes in cell membrane

could be attributed to the modification of the distribution of charged species, thus affecting its normal function, and not to poration, given the maximal field strengths applied of 10 V m^{-1}. Furthermore, the PEF treatment conditions that were found to elicit the promotion of the phenolic content in apple fruits are in the range of those used by other authors to promote the synthesis of secondary metabolites in different plant tissues. Our results are in accordance with those previously reported by Vallverdú-Queralt et al. (2012), who observed an increase of up to a 44.6 % of the total phenolic content in tomato fruits subjected to PEF treatments of similar intensity and left for 24 h at 4 ºC. The accumulation of extracellular phenolics was induced in cell cultures of grape following the application of 10 pulses of 1.2 kV cm^{-1} (Cai et al, 2010). The same conditions were reported to produce a 25 % increase in the amount of anthocyanins accumulated in a grape cell culture in the following 4 d period. Similarly, Gueven and Knorr (2010) reported an increase in isoflavonoid concentration in a soy plant suspension culture subjected to PEF treatments with an intensity of 0.2 kV cm^{-1}. To the best of our knowledge, no literature works are available reporting on the effect of PEF on phenolic compounds in apple tissues. Nevertheless, the effect of electric fields on the respiratory response of apple, an indicator of the overall response of the fruit to oxidative stress, has been previously reported. Atungulu et al. (2003) observed the retardation of respiration and suppression of climacteric peak in apples subjected to an electrostatic field. A similar reduction of the respiratory response and ethylene production is described by Wang et al. (2008) for tomato fruits subjected to an electrostatic field, whose extent is associated with the ability of the treatments to modulate the fruit metabolic response. However, the treatment conditions in the previously cited works substantially differs from those applied in the present study, as a

different type of electrical stimulus (continuous electric field) and way of application (electrodes separated by air) were used.

It is as well worth noticing that the rates of increase of flavan-3-ol compounds after the treatments were much greater than those observed for other compounds and that there was a significant influence of temperature on the PEF-elicited response. The biochemical mechanism by which biosynthetic pathways are selectively activated exceeds the scope of this study and requires more profound studies on the metabolic profile. However, as reported for other well studied elicitors, plant cells can perceive a stress factor in many ways and have numerous networks for transducing the generated signals and there exists elicitor specificity respect to the activated signal components (Kaimoyo et al., 2008; Vasconsuelo and Boland, 2007); hence, the selectivity of the response of the fruit tissue to PEF would depend to a great extent on the pathways activated, which would in turn depend on the nature of the changes generated by the treatments at a cellular level.

5. Conclusions

PEF treatments can be applied to elicit an increase of the antioxidant potential in apple fruits. The stimulation the secondary metabolism of the fruits can be optimized under selected PEF conditions in order to enhance the accumulation of phenolic compounds in the plant tissue. A proper combination of PEF, storage time and temperature is crucial to achieve positive effects. Apples subjected to PEF treatments delivering an overall energy input of 0.008 kJ kg^{-1} and stored for 24 h at 22 ºC h exhibited the highest increase in their phenolic content. Nevertheless, the greatest absolute increase in overall

antioxidant capacity values was reached when storing the fruits at 4 ºC for 12 h. The extent of the observed changes depended on the nature of the phenolic compounds at stake, thus revealing the specificity of PEF when applied as an abiotic source of stress. Further studies focussing on the effects of PEF on the fruit metabolism and structure should be carried out in order to gain knowledge regarding the processes associated to the changes in the antioxidant potential of apple fruits. These results confirm the potential of PEF treatments as a feasible strategy for promoting the antioxidant potential in raw materials prior to processing with the aim of developing healthier apple-based products.

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485 **Figure Captions**

486

487 Figure 1.- Effects of PEF treatment intensity and storage time on the total phenolic
488 content of apples stored at 4 ºC (A) and 22 ºC (B). Data represents the mean and
489 standard deviation (n=6). Total phenolic content was expressed as g kg^{-1} of gallic acid
490 equivalents (GAE).

491

492 Figure 2.- Effects of PEF treatment intensity and storage time on the flavonoid content
493 of apples stored at 4 ºC (A) and 22 ºC (B). Data represents the mean and standard
494 deviation (n=6). Flavonoid content was expressed as g kg^{-1} of (+)-catechin equivalents
495 (CE).

496

497 Figure 3.- Effects of PEF treatment intensity and storage time on the flavan-3-ol content
498 of apples stored at 4 ºC (A) and 22 ºC (B). Data represents the mean and standard
499 deviation (n=6). Flavan-3-ol content was expressed as g kg^{-1} of (+)-catechin equivalents
500 (CE).

501

502 Figure 4.- Effects of PEF treatment intensity and storage time on the antioxidant
503 capacity of apples stored at 4 ºC (A) and 22 ºC (B). Data represents the mean and
504 standard deviation (n=6). Antioxidant capacity was expressed as mmol kg^{-1} of trolox
505 equivalents (TE).

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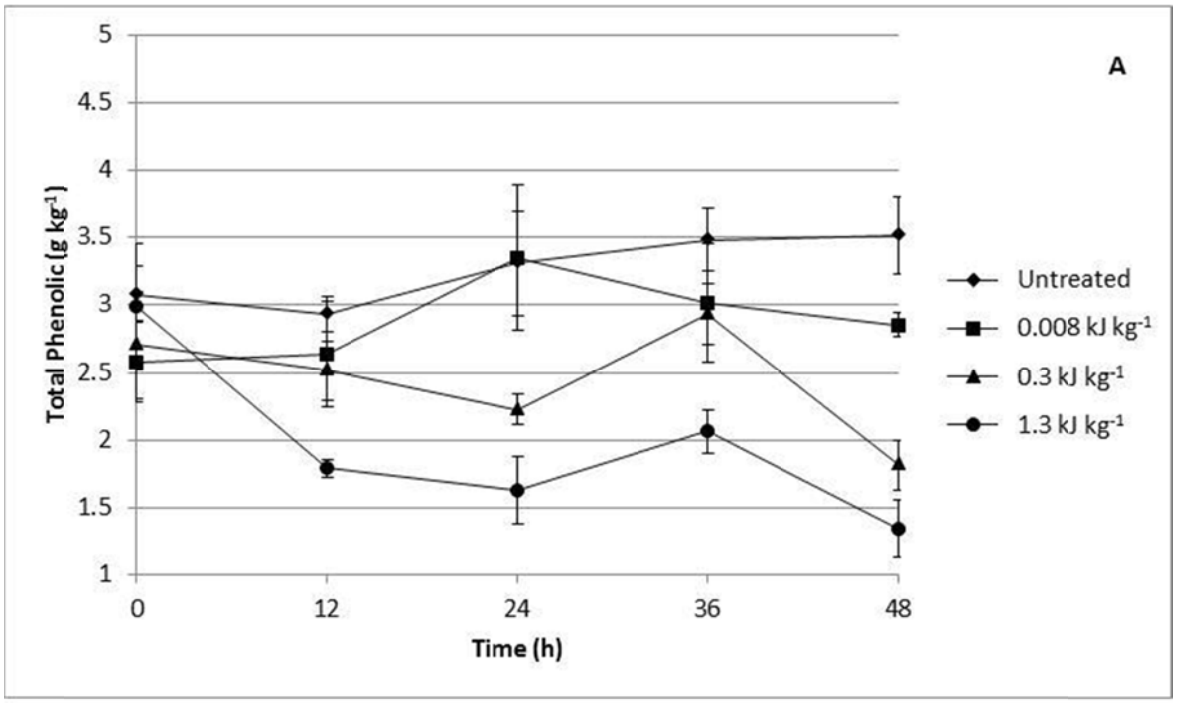

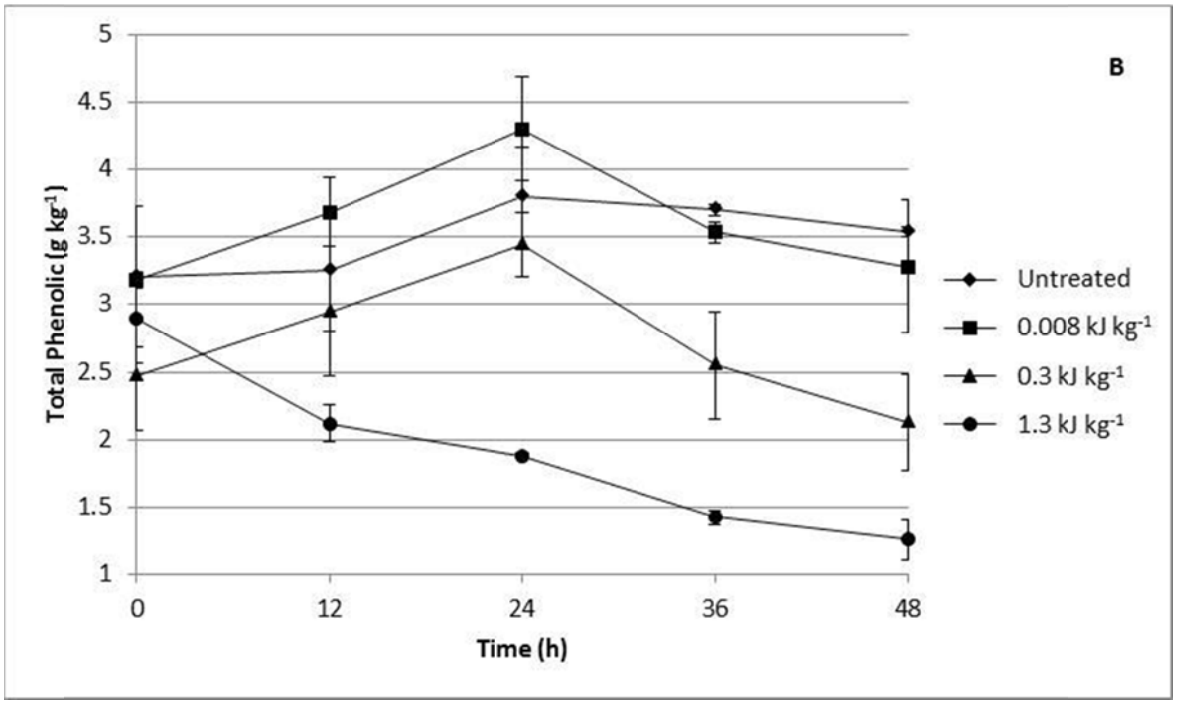

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512 Figure 1.-

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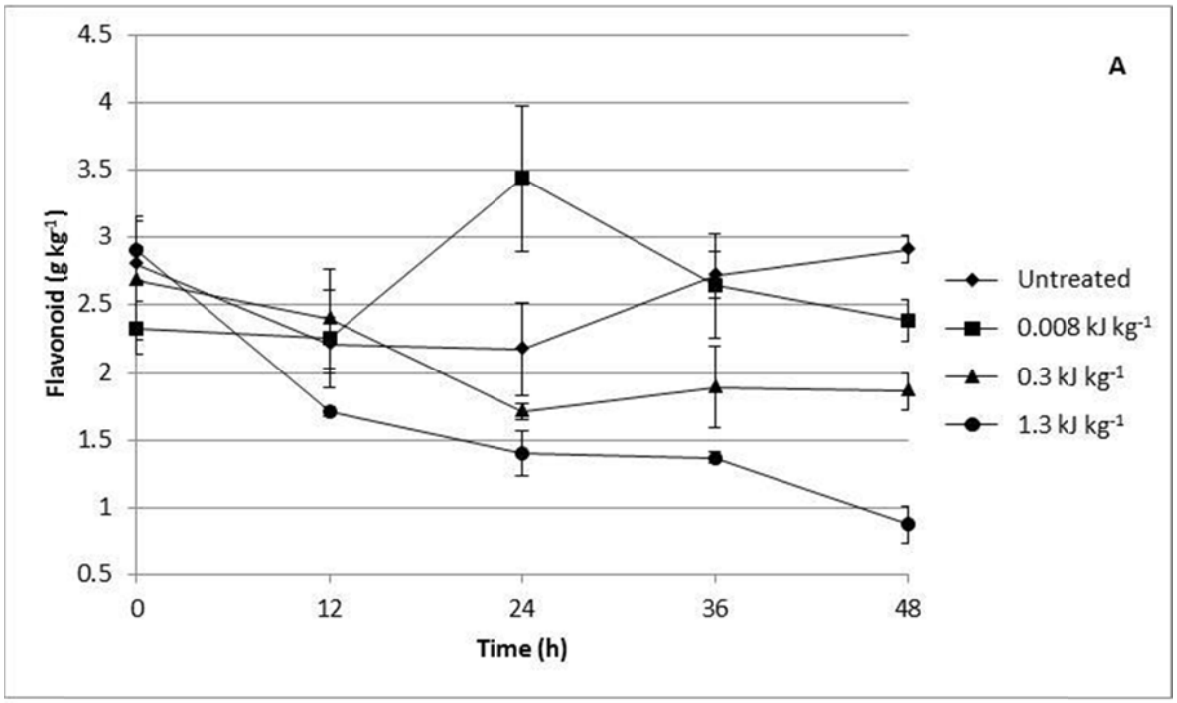

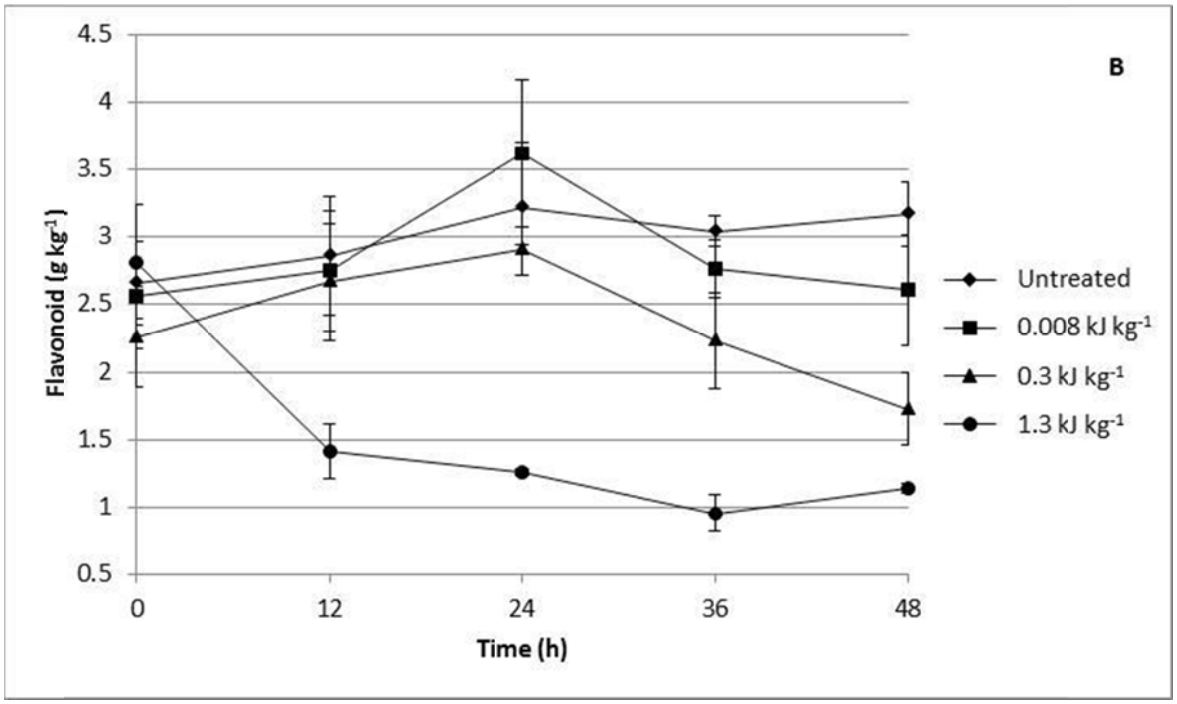

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517 Figure 2.-

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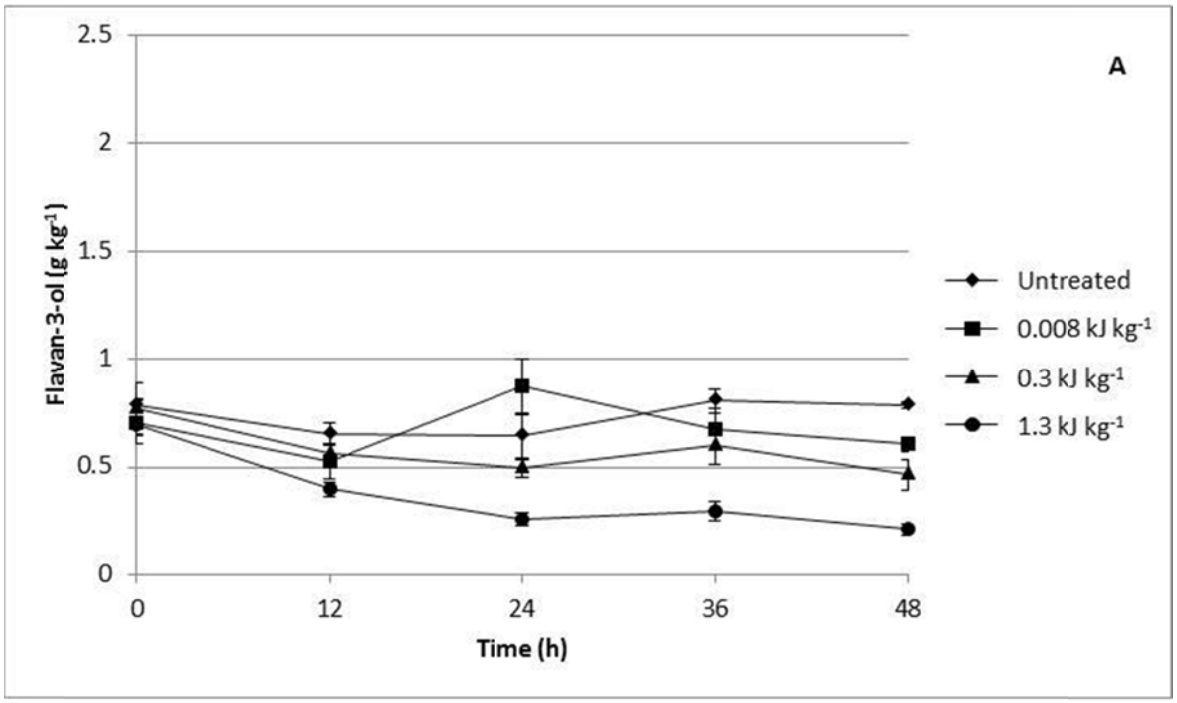

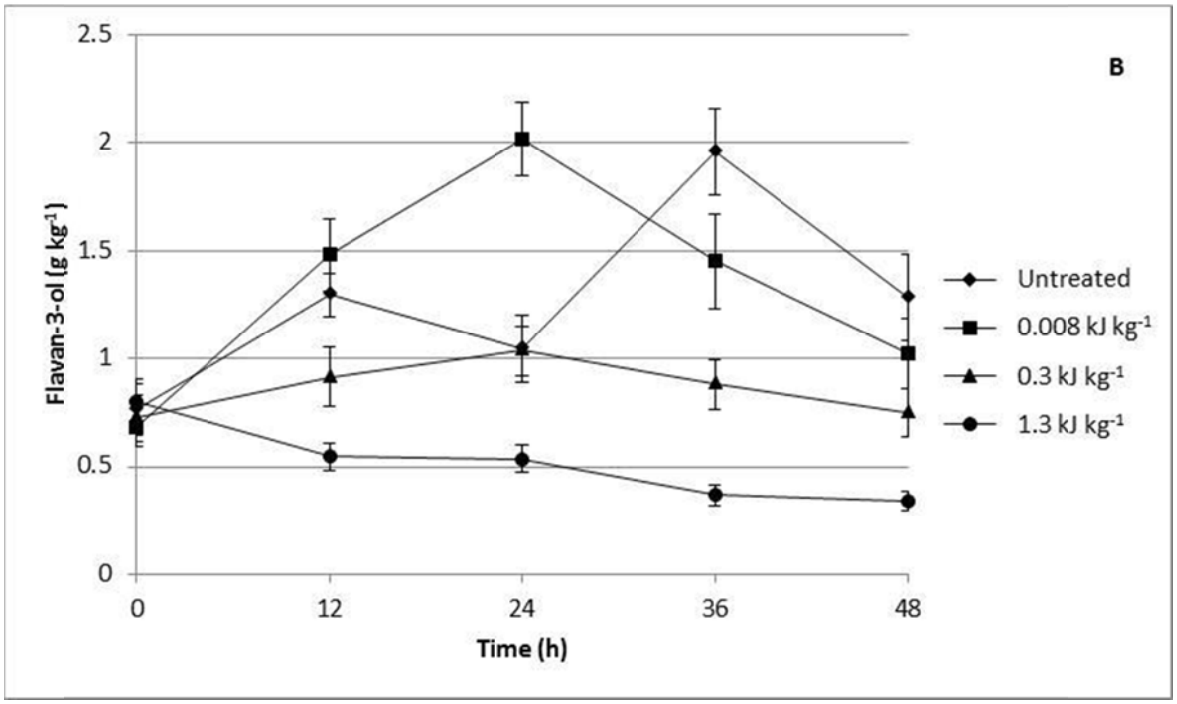

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522 Figure 3.-

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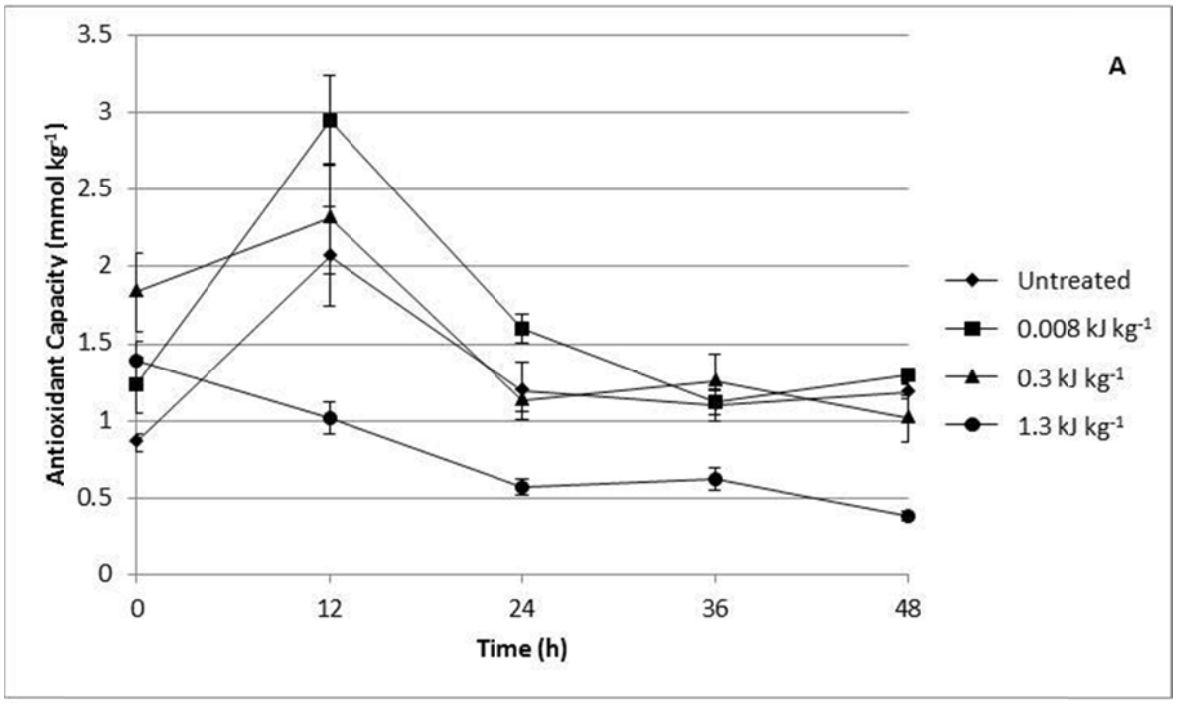

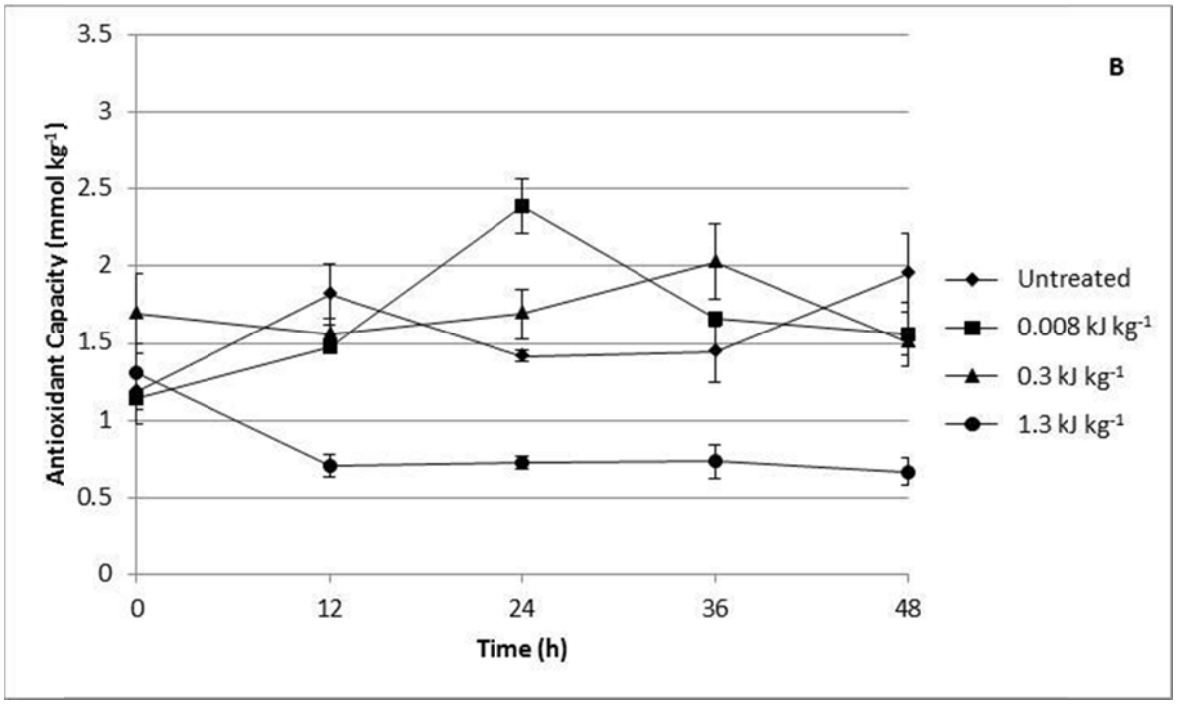

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527 Figure 4.-

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